AD 222 915

FINAL REPORT

May 1, 1954

Office of Naval Research, N7onr-410/I, NR-130-391
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The Cellular Reactions Associated with Streptococcal Infections

Studies on the cellular reactions mentioned in the previous progress report have been completed. It has been confirmed that the first cells which appear in the lungs after the intratracheal injection of Group A, Type 3 Strep. progenes are histiocytes. These are by far the predominant cell type in the first 2 to 3 hours after injection. All of the phagocytosis of Streptococcus in the early part of the infection is carried out by histiocytes. Neutrophiles, which become predominant 3 to 4 hours following injection are only moderately active later in the course of the injection. These studies also showed that the cell responsible for the final cleaning up of the infection is the histiocyte. These results were obtained in the hamster lung, and confirm completely studies of the same type carried out 4 years ago. In order to determine whether the cellular response observed was a particular feature of the hamster or of pulmonary tissue, infections with the same type of beta-hemolytic streptococcus were produced in the skin of rabbits, guinea pigs and hamsters, in the thigh muscles of hamsters, guinea pigs and rabbits and in the lungs of guinea pigs and rabbits. Tissues were removed at 10 minutes, 1/2 hour, 1 hour, 4, 8, 12, 24, 48, 72 and 168 hours after infection and the cellular reactions studied, after proper staining which demonstrated bacteria as well as cell type. The reactions in the lungs of the hamster, guinea pig and rabbit were identical. The first cell to appear in the skin and muscles was the neutrophile and all of the phagocytosis appeared to be carried out by this cell. The histiocyte was of importance in streptococcal infection of these tissues mainly in the late stages when it appeared to appear in large numbers to clean up necrotic debris. The studies indicate that an early outpouring of histiocytes and active phagocytosis of streptococci by this cell type is a characteristic of pulmonary infection and that it is not associated with any particular species of animal. This work has now been completed and the results are now being prepared for publication.

Studies with Fractions of the Beta-hemolytic Streptococcus

Two groups of fractions were prepared from the cellular contents of Type 3 beta-hemolytic Streptococcus according to methods previously

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described and labeled P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10 and A, B, C, D, E, F, and G. These fractions were prepared by precipitation using different ph levels and ionic strengths of sodium chloride as well as varying concentrations of ethyl alcohol. In addition, extracts of dried streptococcal cells in large quantity were made using ethyl ether, chloroform and petroleum ether. These 20 fractions were studied by various methods.

1. The Injection of Streptococcal Fractions into Animals

All of the 20 fractions mentioned above were injected into animals and the tissue reactions studied using sterile saline as control material for the P and A-G fractions and sterile oils as control for the fats extracted from the Streptococcus. No specific cellular reactions were produced in skin, lungs, or muscle tissue following injection of the various fractions. Although the streptococcal fats produced necrosis of the skin of rabbits and guinea pigs when injected endermally, study of the cellular reactions microscopically revealed no essential difference from those observed when sterile mineral, peanut or sesame oils were injected.

All of the P, A-G, and fat extracts were injected in varying quantities, into the tail vein of mice, some animals receiving a single injection while others were given several injections at weekly intervals. At periodic intervals after injection, the mice were sacrificed and the heart and kidneys studied microscopically, after proper staining. No specific lesions were noted in any of these animals with the occasional exception of interstitial collection of lymphocytes and histiocytes. These lesions were not constant and were noted, furthermore, in control mice which had received 0.85 per cent saline intravenously.

2. The Injection of Streptococcal Fractions into Embryonated Eggs

All of the P, A-G and fat fractions have been injected in known quantities into embryonated eggs and the mortality rates studied. Twenty eggs have been injected with each fraction up to the present and the results obtained suggest that certain fractions produce death of the embryo while others do not appear to interfere with development. Some of the chicks developing from embryos which had been exposed to various of the streptococcal fractions were born with deformed feet suggesting the presence of an arthritis; this phenomenon was not observed in chicks developing from "control eggs". This work has not yet been completed and is still being carried out. It is planned to inoculate at least 100 eggs with each fraction in order to obtain data which are statistically significant when compared to controls.

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The point changes and duration of life of chicks developing from eggs inoculated with non-lethal fractions is also still being studied at present.

3. Immunization of Rabbits with Streptococcal Fractions

All of the P and A-C fractions have been inoculated into the ear veins of rabbits repeatedly. Control animals received saline intravenously while another group of rabbits was injected with suspensions of dead, whole streptococci of the same type as that from which the fractions were prepared. Serums were collected at periodic intervals during the period of immunization which was carried on for 6 months. Precipitating antibody filtrations against the various fractions were carried out using the Lancefield microprecipitin technic. The presence of antibody was also studied by a method involving the coating of red blood cells with the fractions of the streptococcus and exposing these coated cells to each of the rabbit sera in the absence or presence of complement. Four of the P fractions and 2 of the A-C fractions were found to be antigenic by the precipitation test. In all instances, however, sera obtained late in the course of immunization appeared to have lost the ability to precipitate the specific antigen. Serum obtained from rabbits immunized with whole dead streptococci contained antibody for one of the A-C and 5 of the P fractions. In no instance was any cross-reaction observed in the sera produced by immunization with fractions. In the absence of complement, no reaction was observed when red blood cells coated with fractions were exposed to the specific sera. In the presence of complement, hemolysis of coated red blood cells occurred with most of the sera suggesting a high degree of cross-reactivity between the fractions, although this is probably a highly sensitive method and open to some question in relation to non-specific cross-reactivity. The sera obtained following immunization with whole, dead streptococci contained large quantities of precipitating antibody for highly purified type specific nucleoprotein (Type 3M). This work is being continued.

4. Antibodies to Streptococcal Fractions in Human Sera

The serums of 100 patients with rheumatic fever (active and inactive), 100 patients with scarlet fever (acute and late convalescent stages) and 200 people with a variety of infectious and non-infectious disease were examined for precipitating antibody to all of the P and A-C fractions using the micro-precipitin technicof Lancefield. All of the fractions were first examined for the presence of the Type 3 specific M and were found to contain this nucleoprotein. When tested with type-specific sera against most of the other serological types of streptococci, no M substance other than type 3 was found.

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Since, as noted below, some of the human serums examined revealed precipitin for a broad variety of types (relatively infrequently to type 3) while others contained no antibody for any of the M substances of the Streptococcus, it may be concluded that the precipitation observed when human sera and streptococcal fractions were mixed resulted probably from a specific antigen-antibody reaction and was not due merely to non-specific reaction with the M substance contained in the fractions. As noted in the previous progress report 65% of the rheumatic fever patients, 20% of those with scarlet fever and only 5% of those who had neither of these diseases showed precipitation when their serum was mixed with one of the P fractions. A similar preponderance of reactivity was noted with rheumatic fever serum and one of theA-C fractions. Plotting of the precipitin reactions against the fractions used suggests that 3 patterns of reactivity with the P fractions used be associated with the diseases studied. Thus, with rheumatic fever the highest order of reactivity is in the range of fractions P1, P2 and P3, with scarlet fever sera it is mainly P3 and P4, while the normal sera the entire range of reaction is low with all fractions. These data suggest the possibility of studying rheumatic fever (using scarlet fever and other infectious disease patients as controls) for the presence of this type of precipitating reaction in the hope that the confirmation of a specific pattern of reactivity with the P fractions may be helped in establishing diagnosis of this disease. This work will be extended during the next few years to include study of sera obtained during various stages of rheumatic activity and sera obtained from patients with uncomplicated streptococcal infection as well as other diseases.

III. Studies of Antibodies to Various Specific Nucleoproteins (M) of the Streptococcus in Human Blood

These studies, mentioned in last year's progress report, have now been completed and the results are in the process of being gathered and tabulated for publication. It has been found that the serums of rheumatic fever patients contain precipitating antibody to anywhere from 5 to 20 of the specific serologic types. The serums of scarlet fever patients, on the other hand, react with few M's while those of normal people react to even a smaller extent. It is planned to extend these studies in the future to include several thousand people from various parts of the world and to study the distribution of anti-M antibody as related to age.

Studies on the relationship of the presence of anti-M precipitating antibody in the serum and the bactericidal potency against the same serological type of Streptococcus have been completed. No correlation could be established between the presence of precipitating antibody for the type - specific nucleoprotein of a strain of Streptococcus and

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the ability of the serum to kill organisms of the same serological type. This may be due to the low titer of precipitating antibody noted in most of the serums.

IV. Preparation of Strains of Streptococci Virulent for Mice and Vibration of These Strains for the Purpose of Producing Immunizing Materials

Thirty-two different serological types of beta-hemolytic Streptococcus, none of which were virulent for white mice, have been inoculated into these animals, recovered and passed into new animals repeatedly for the last one and one-half years. Twenty of these strains now kill mice in 18 hours after intraperitoneal injection of 0.1 ml. of a 10^{-4} to 10^{-6} dilution. Seven strains kill only when given in a dilution of 10^{-2} to 10^{-3} while the other 5 strains have shown little or no increase in virulence.

All of these strains of Streptococci have been grown in large quantities and subjected to sonic vibration. Preparations from these vibrated streptococci will be used in various forms in an attempt to immunize rabbits at first, and later humans in order to study the bactericidal capacity of the serum and its duration. Studies are also planned to determine whether any cross-reaction in bactericidal effects occurs among the various types.

V. Electrophoresis of Streptococcal Fractions

Two of the 3 fractions have been subjected to study in the Tiselius electrophoresis apparatus up to the present. These show quite distinct patterns suggesting a mixture of several components rather than a single substance. This study has just been initiated and will continue for at least the remainder of the year.

VI. The Production of Cardiac Lesions in Guinea Pigs

The work of Murphy and Swift in which cardiac lesions resembling those of rheumatic fever were produced in rabbits by repeated infection of the skin with different serological types of beta-hemolytic Streptococcus is being repeated. The guinea pig is being used because of the relative ease with which this animal can be sensitized. Immunologic and pathologic studies of the same type carried out by Murphy and Swift will be done on the guinea pigs. This work has just been started and will probably require a year to complete.

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